



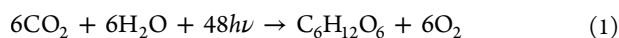
Photoreduction of CO₂ with a Formate Dehydrogenase Driven by Photosystem II Using a Semi-artificial Z-Scheme Architecture

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Supporting Information

ABSTRACT: Solar-driven coupling of water oxidation with CO₂ reduction sustains life on our planet and is of high priority in contemporary energy research. Here, we report a photoelectrochemical tandem device that performs photocatalytic reduction of CO₂ to formate. We employ a semi-artificial design, which wires a W-dependent formate dehydrogenase (FDH) cathode to a photoanode containing the photosynthetic water oxidation enzyme, Photosystem II, via a synthetic dye with complementary light absorption. From a biological perspective, the system achieves a metabolically inaccessible pathway of light-driven CO₂ fixation to formate. From a synthetic point of view, it represents a proof-of-principle system utilizing precious-metal-free catalysts for selective CO₂-to-formate conversion using water as an electron donor. This hybrid platform demonstrates the translatability and versatility of coupling abiotic and biotic components to create challenging models for solar fuel and chemical synthesis.

In the thylakoid membrane of plants, light-driven water oxidation in the photosynthetic Z-scheme is coupled to CO₂ fixation for sugar synthesis via the dark Calvin–Benson–Bassham (CBB) cycle (eq 1).^{1,2} Although this solar-energy-



storing reaction is one of the most fundamental processes in biology and essential for life, it also exemplifies the inefficiencies of solar-to-fuel conversion.³ For example, Photosystem II (PSII) and Photosystem I (PSI) are non-complementary light absorbers, which limits light harvesting efficiency. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is responsible for CO₂ fixation but has low turnover rates (1–10 s^{−1}), thereby creating a significant kinetic bottleneck. RuBisCO also reacts with O₂ to produce 2-phosphoglycolate, which must be recycled in energy-demanding, CO₂-evolving photorespiration.^{4,5} The CBB cycle involves

significant adenosine triphosphate (ATP) consumption, which leads to a lower biomass production efficiency compared to the prokaryotic reductive acetyl-coenzyme A (rAcCoA) pathway.⁶ This alternative, light-independent route to CO₂ fixation uses the energy vector hydrogen as electron donor to reduce two CO₂ molecules to acetate in a linear sequence of reaction steps.⁷

Addressing the limitations of biological carbon fixation presents several challenges,^{8–14} leading research toward *in vitro* (but light-independent) carbon fixation pathways.¹⁵ As a bio-inspired alternative, artificial photosynthesis aspires to couple solar-light-driven water oxidation with CO₂ reduction to chemical fuels at higher efficiency than natural systems.¹⁶ However, artificial photosynthetic carbon fixation is currently not economically feasible due to a lack of efficient, selective, or inexpensive catalysts and light absorbers.¹⁷

One of the entry points of CO₂ into the rAcCoA pathway is its conversion to formate before transfer to tetrahydrofolate (the second entry point involves its reduction to CO by carbon monoxide dehydrogenase/AcCoA synthase).⁷ Coupling this process to light-driven water oxidation is a compelling step toward creating an efficient, artificial photosynthetic carbon fixation pathway. Formate is also a stable intermediate between CO₂ and methanol/methane, a hydrogen carrier, and a viable fuel itself.^{18,19} Semi-artificial photosynthesis, in which catalytically efficient redox enzymes are interfaced with synthetic materials, offers a possibility to couple this key entry point of the rAcCoA pathway to light-driven CO₂ reduction and bypasses the energy-demanding and inefficient use of ATP.

Mo- and W-dependent formate dehydrogenases (FDHs) are enzymes capable of interconverting CO₂ and formate.^{20–28} When adsorbed on an electrode, FDHs from *Syntrophobacter fumaroxidans*²¹ and *Escherichia coli*^{24,28} have been shown to perform reversible electrocatalysis with high efficiency through fast interfacial electron transfer. The activity of a Mo-FDH from *E. coli* has been harnessed in fuel cell devices, in which it was immobilized in cobaltocene- and viologen-functionalized

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redox polymers.^{29,30} Electrochemical CO₂ reduction using a W-FDH has been reported in mediated^{31,32} and unmediated systems.²⁷ These FDHs contrast with metal-independent FDHs, which reduce CO₂ using nicotinamide adenine dinucleotide (NADH), an unstable, expensive, and diffusive cofactor with little driving force.^{33–42} Metal-independent FDHs have been coupled to molecular,^{43–46} biological,^{47,48} and solid-state^{38,41} visible-light-absorbers. In addition to the limitations of NADH utilization, these systems suffer from low selectivity and rely on sacrificial electron donors.

Here, we report a semi-artificial photoelectrochemical (PEC) tandem cell that wires the enzymes PSII and FDH to perform light-driven CO₂ conversion to formate using water as an electron donor (eq 2). First, we study the CO₂ reduction



activity of W-FDH from *Desulfovibrio vulgaris*⁴⁹ adsorbed on a hierarchically structured inverse opal titanium dioxide (IO-TiO₂) scaffold (IO-TiO₂|FDH). This IO-TiO₂|FDH cathode is then wired to a recently reported PSII-based dye-sensitized photoanode, IO-TiO₂|dpp|P_{Os}-PSII,⁵⁰ which combines isolated PSII from *Thermosynechococcus elongatus*, dpp (a phosphonated diketopyrrolopyrrole dye), and P_{Os} [poly(1-vinylimidazole-co-allylamine)-[Os(bipy)₂Cl]Cl redox polymer] to realize a light-driven rAcCoA pathway by coupling selective CO₂ fixation to light-driven water oxidation (Figure 1).

In this enzyme-catalyzed PEC system, photogenerated electrons in PSII, which is embedded in the redox polymer P_{Os}, are transferred to the electron acceptor plastoquinone B

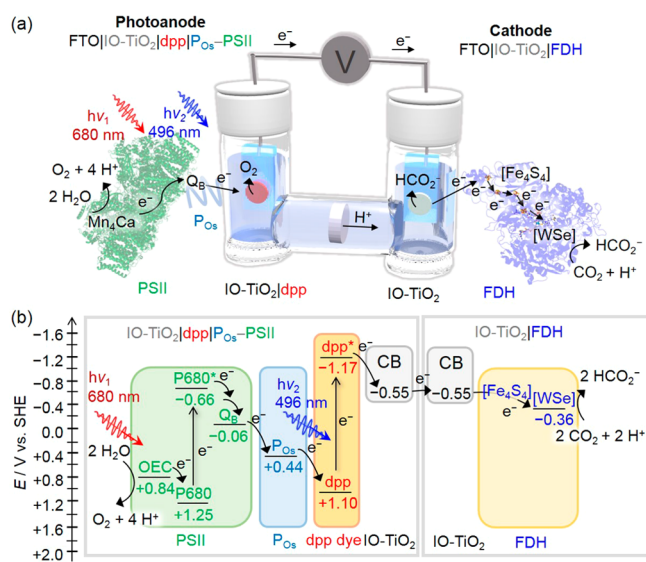


Figure 1. (a) Schematic representation of the semi-artificial photosynthetic tandem PEC cell coupling CO₂ reduction to water oxidation. A blend of P_{Os} and PSII adsorbed on a dpp-sensitized photoanode (IO-TiO₂|dpp|P_{Os}-PSII) is wired to an IO-TiO₂|FDH cathode (species size not drawn to scale). (b) Energy level diagram showing the electron-transfer pathway between PSII, the redox polymer (P_{Os}), the dye (dpp), the conduction band (CB) of IO-TiO₂ electrodes, four [Fe₄S₄] clusters, and the [WSe]-active site in FDH. All potentials are reported vs SHE at pH 6.5. Abbreviations: Mn₄Ca, oxygen-evolving complex (OEC); P680, pigment/primary electron donor; Q_B, plastoquinone B; [Fe₄S₄], iron–sulfur clusters; [WSe], FDH active site.

(Q_B, Figure S1). The holes are collected at the oxygen-evolving complex (OEC), where water is oxidized to liberate protons and gaseous O₂. The Os³⁺ complex in P_{Os} mediates electron transfer between reduced Q_B and oxidized dpp⁺. The conduction band (CB) of IO-TiO₂ receives electrons from the photoexcited dpp*. Electrons are transferred through the external electrical circuit to the IO-TiO₂|FDH cathode and arrive at the CO₂-reducing [WSe]-active site via interfacial electron transfer from the TiO₂ CB to iron–sulfur clusters (Fe₄S₄) which connect the FDH active site to its surface.

Hierarchical macro-mesoporous IO-TiO₂ electrodes (20 μm film thickness; geometrical surface area, A = 0.25 cm²) were assembled on a fluorine tin oxide (FTO)-coated glass substrate (see Supporting Information).⁵⁰ An FDH solution (2 μL, 17 μM with 50 mM DL-dithiothreitol, incubated for 10 min) was drop-cast onto IO-TiO₂ to give the IO-TiO₂|FDH cathode. Anaerobic conditions were employed due to possible O₂ inhibition of FDH and side reactions of the electrode components with O₂. Protein film voltammetry (PFV) of IO-TiO₂|FDH in a solution of CO₂/NaHCO₃ (100 mM, pH 6.5, under 1 atm CO₂) and KCl (50 mM) demonstrated the high CO₂ reduction activity of the electrode (Figure 2). The current density (J) of IO-TiO₂|FDH was measured as a function of an applied potential (E_{app}) in a three-electrode configuration. The onset potential for CO₂ reduction to formate was observed close to the thermodynamic potential of the CO₂/HCO₂[−] couple (−0.36 V vs standard hydrogen electrode, SHE) at approximately −0.4 V vs SHE, and a current density of −240 μA cm^{−2} was reached at −0.6 V vs SHE.

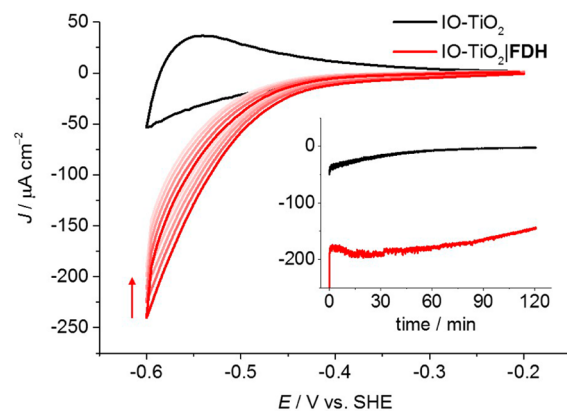


Figure 2. PFV scans ($\nu = 5 \text{ mV s}^{-1}$) of IO-TiO₂ (black trace) and IO-TiO₂|FDH (red traces, arrow indicates scan order). Inset: CPE at E_{app} = −0.6 V vs SHE. Conditions: CO₂/NaHCO₃ (100 mM), KCl (50 mM), 1 atm CO₂, pH = 6.5, T = 25 °C, continuous stirring. The three-electrode configuration employed a two-compartment cell with Ag/AgCl (saturated KCl) reference and Pt mesh counter electrodes.

The IO-TiO₂|FDH electrode exhibited good stability, retaining approximately 83% of its initial activity after controlled-potential electrolysis (CPE) for 2 h at E_{app} = −0.6 V vs SHE (Figure 2, inset). The Faradaic efficiency (η_F) of formate production was determined as (78 ± 8)% (2.22 ± 0.23 μmol cm^{−2}). A voltammogram recorded immediately after the CPE experiment indicated electrode behavior similar to that measured before CPE (Figure S2), though with slightly lower, yet stable, activity. No H₂ production was detectable by gas chromatography (GC) analysis of the cell headspace, suggesting that the background current was due to charging

of the CB of TiO_2 (Figure 2).⁵¹ The relatively high current densities of the IO- TiO_2 /FDH electrode were likely due to high enzyme loading and effective wiring inside the porous, hierarchically structured IO- TiO_2 scaffold.^{52,53} Thus, the cathode proved to be suitable for coupling to PSII-catalyzed water oxidation in a two-electrode PEC setup.

The activity of the IO- TiO_2 /dpp/ P_{O_8} -PSII electrode in CO_2 /NaHCO₃/KCl electrolyte solution was measured by stepped-potential chronoamperometry under periodic simulated solar illumination (Figure S3), showing behavior comparable to that of the recently reported PSII-modified dye-sensitized photoanode.⁵⁰ The photoanode was electrically wired to the IO- TiO_2 /FDH cathode via a potentiostat, and the two electrodes were placed in compartments separated by a glass frit membrane in a PEC cell (Figure 1).

Stepped-voltage chronoamperometry under periodic illumination with UV-filtered simulated solar light (AM1.5G; irradiance $E_e = 100 \text{ mW cm}^{-2}$; $\lambda > 420 \text{ nm}$, Figure 3a) was used to study the system's performance. Upon irradiation, a current density of $5.5 \pm 0.4 \mu\text{A cm}^{-2}$ was observed at zero applied voltage (U_{app}) (Figures S4 and S5). Voltage-

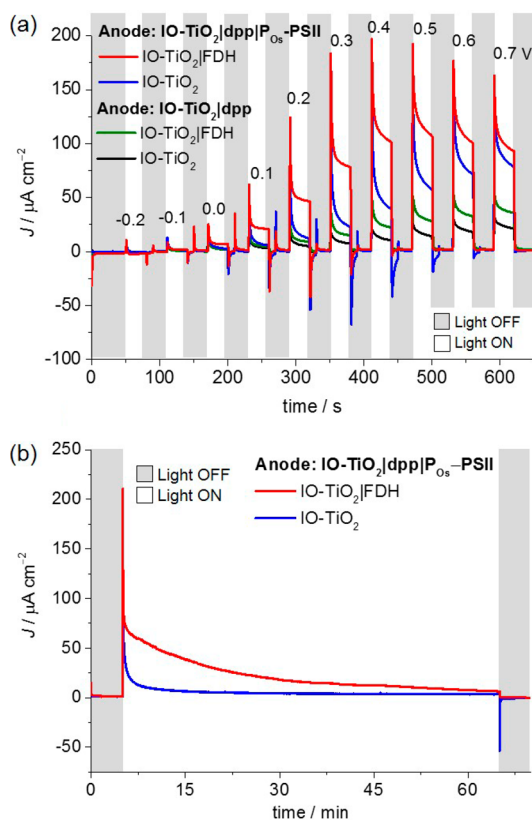


Figure 3. Characterization of two-electrode PEC cell consisting of IO- TiO_2 /FDH cathode wired to IO- TiO_2 /dpp/ P_{O_8} -PSII tandem photoanode. (a) Representative stepped-voltage chronoamperometry (0.1 V voltage steps with 30 s dark and 30 s light cycles) of the fully assembled PEC cell (red trace). Control experiments in the absence of PSII (green and black traces) and without FDH (blue and black traces) are also shown. Applied voltage (U_{app}) values are shown on top of the traces. (b) CPE ($U_{\text{app}} = 0.3 \text{ V}$) of the two-electrode PSII-FDH system (red trace) and a similar system in the absence of FDH (blue trace). Conditions: CO_2 /NaHCO₃ (100 mM), KCl (50 mM), 1 atm CO_2 , pH = 6.5, $T = 25^\circ\text{C}$, continuous stirring. Simulated solar light source: AM 1.5G filter; $E_e = 100 \text{ mW cm}^{-2}$; $\lambda > 420 \text{ nm}$.

independent steady-state photocurrents ($99 \pm 4 \mu\text{A cm}^{-2}$) were reached at $U_{\text{app}} > 0.4 \text{ V}$. Control experiments showed that small background responses were also observed using PSII-free IO- TiO_2 /dpp photoanodes (Figure 3, green and black traces) due to electron transfer from photoexcited dpp to TiO_2 without dye regeneration, resulting in photobleaching.⁵⁰ When FDH was omitted from the system (Figure 3, blue trace), lower photoresponses were observed than in its presence, but the current response was higher than those responses observed in the absence of PSII. This background current is likely due to high capacitance of the high surface area IO- TiO_2 (charging of TiO_2 CB), supported by the cathodic discharging spikes observed upon switching off the light and persisting photocurrents in the chronoamperometry measurements with longer irradiation time (Figure S6). Substantial capacitance currents over a long time scale consistent with those observed in this study have been previously observed for porous TiO_2 electrodes.^{51,54} At lower applied voltages ($U_{\text{app}} < 0.4 \text{ V}$), Faradaic current from CO_2 reduction with FDH and some charging of TiO_2 should dominate, whereas at higher applied voltages ($U_{\text{app}} > 0.5 \text{ V}$), substantial TiO_2 CB charging and possibly electrode degradation (e.g., FTO breakdown) could become significantly competing processes (Figure S7).

Only a small bias was required to drive the overall reaction (eq 2). CPE at $U_{\text{app}} = 0.3 \text{ V}$ with the IO- TiO_2 /dpp/ P_{O_8} -PSII//IO- TiO_2 /FDH PEC cell under illumination was performed (Figure 3b). The photocurrent decayed from 92 to $7 \mu\text{A cm}^{-2}$ after 1 h irradiation with a half-life time ($\tau_{1/2}$) of $\sim 8 \text{ min}$ (Figure S8). Prolonged irradiation resulted in an irreversible drop in photocurrent, most likely due to PSII photodegradation.³ Formate was detected ($0.185 \pm 0.017 \mu\text{mol cm}^{-2}$) with $\eta_F = (70 \pm 6)\%$, but reliable O_2 analysis (estimated $0.132 \mu\text{mol cm}^{-2}$, 0.01% O_2 , assuming quantitative η_F) was prevented by the detection limit of the apparatus. Other products such as H_2 and CO could not be detected in the cathodic chamber. No products (H_2 , CO, and formate) were observed in control experiments omitting FDH at $U_{\text{app}} = 0.3$ and 0.6 V (Figures 3b and S7).

In summary, we have demonstrated that the IO- TiO_2 /dpp/ P_{O_8} -PSII//IO- TiO_2 /FDH PEC cell achieves the biologically and synthetically challenging coupling of solar-driven water oxidation to selective CO_2 reduction with a small additional supply of energy (applied voltage) under mild conditions. The semi-artificial architecture employs efficient enzymes and synthetic components that enable not only complementary light absorption but also the coupling of unnatural redox partners, which is challenging *in vivo*. The PSII-FDH tandem PEC system reported here demonstrates how semi-artificial photosynthesis is a translatable and versatile platform, allowing a variety of electroactive enzymes to be studied electrochemically to gain a better understanding of their activity *in vitro*. From a biological perspective, this system can be viewed as an effective model for an engineered light-driven rAcCoA pathway that bypasses limitations of the natural Z-scheme and CBB cycle. Further biologically relevant electrochemical reactions and redox proteins may be coupled using this approach to introduce a plethora of model systems which extend solar-driven CO_2 reduction to production of value-added chemicals.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b10247.

Materials and experimental methods for the electrode preparation, electrochemistry measurements (PFV, CPE, and PEC), and product analysis, including Figures S1–S8 (PDF)

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Notes

The authors declare no competing financial interest. Additional data related to this publication are available at the University of Cambridge data repository (<https://doi.org/10.17863/CAM.32922>).

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